FiGURE 1

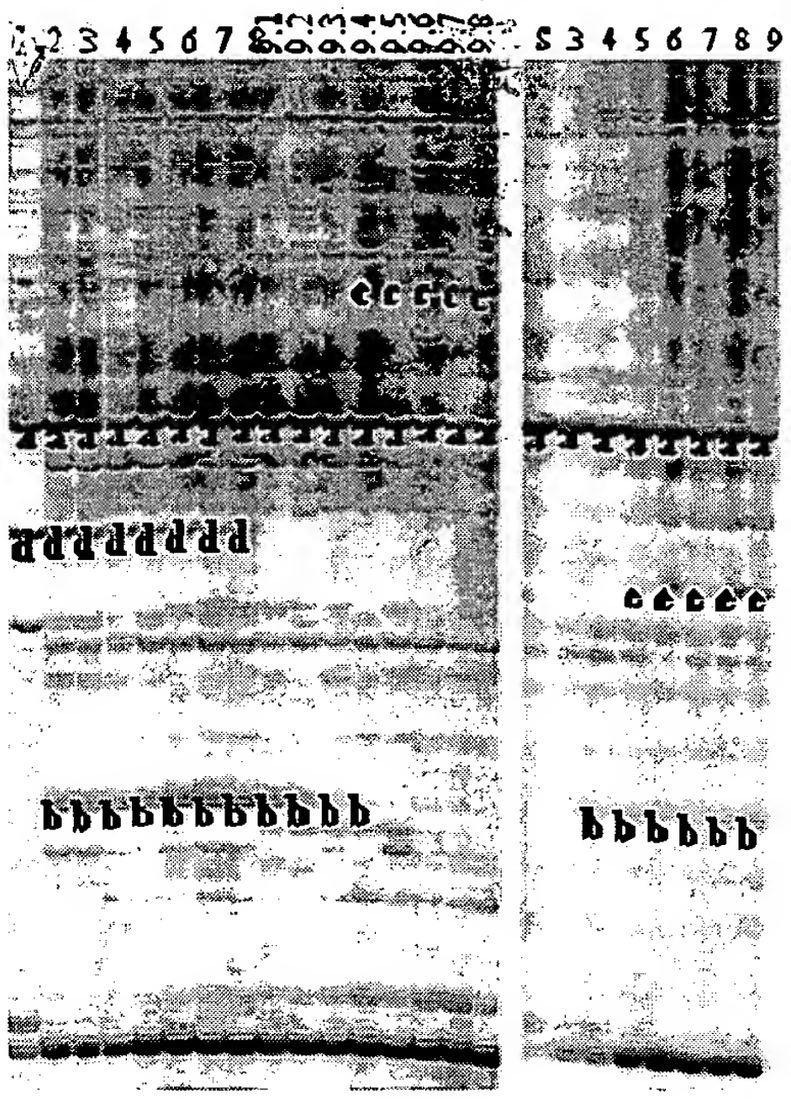


Figure 1. Differential display of loblolly pine zygotic and somatic embryos at different stages of development. The zygotic embryos (left panel) used were from tree BC-1 and the somatic embryos (right panel) are of genotype 260. Primer pair T12VC-AP3 (GenHunter, Nashville, TN) were used in the PCR reactions. The numbers on the top of the lanes indicate the stages of the embryos used. The letters superimposed on the images mark different types of banding patterns: (a), the band appeared in both embryos at all the stages; (b), early to middle stages in ZE and middle to late stages in SE; (c), late stages in ZE and absent in SE; (d), early stages in ZE and absent in SE; (e), present in SE but not in ZE.

FIGURE 2

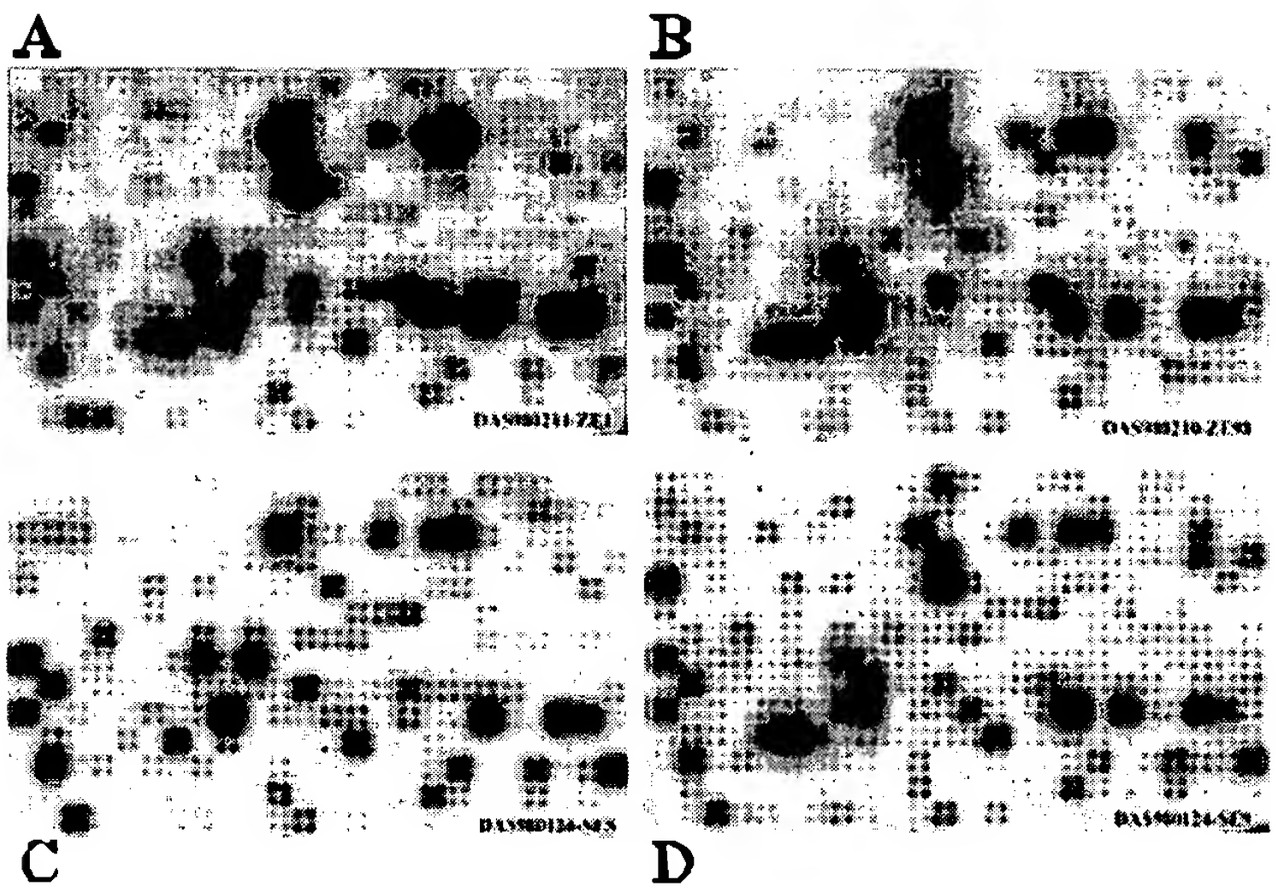


Figure 2. Detection of gene expression by high-density array Southern hybridization. Cloned cDNAs (327) were blotted on a membrane as high-density arrays. Each cDNA was blotted four times as a quadrate. The membranes were hybridized to the total cDNAs derived from total mRNA isolated from zygotic embryos at stage 1 (A), stage 9.8 (B), somatic embryos at suspension stage (C), and stage 9 (D). Dark spots indicate high level of gene expression and light spots indicate low level of gene expression.

FIGURE 3

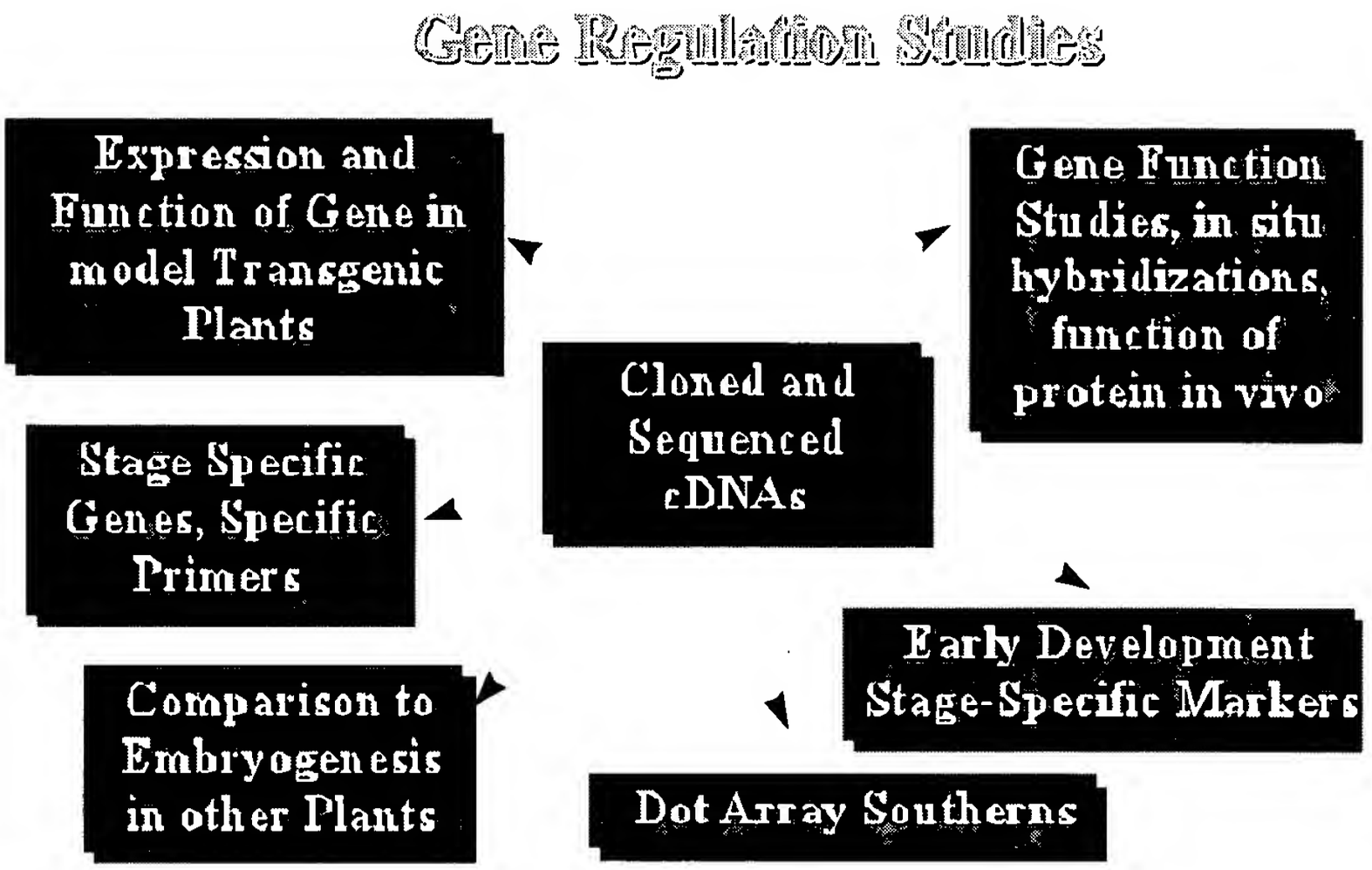


Figure 3. Gene regulation studies arising from the cDNA cloning of genes expressed in embryos. See text for their applicability to process improvement.

FIGURE 4

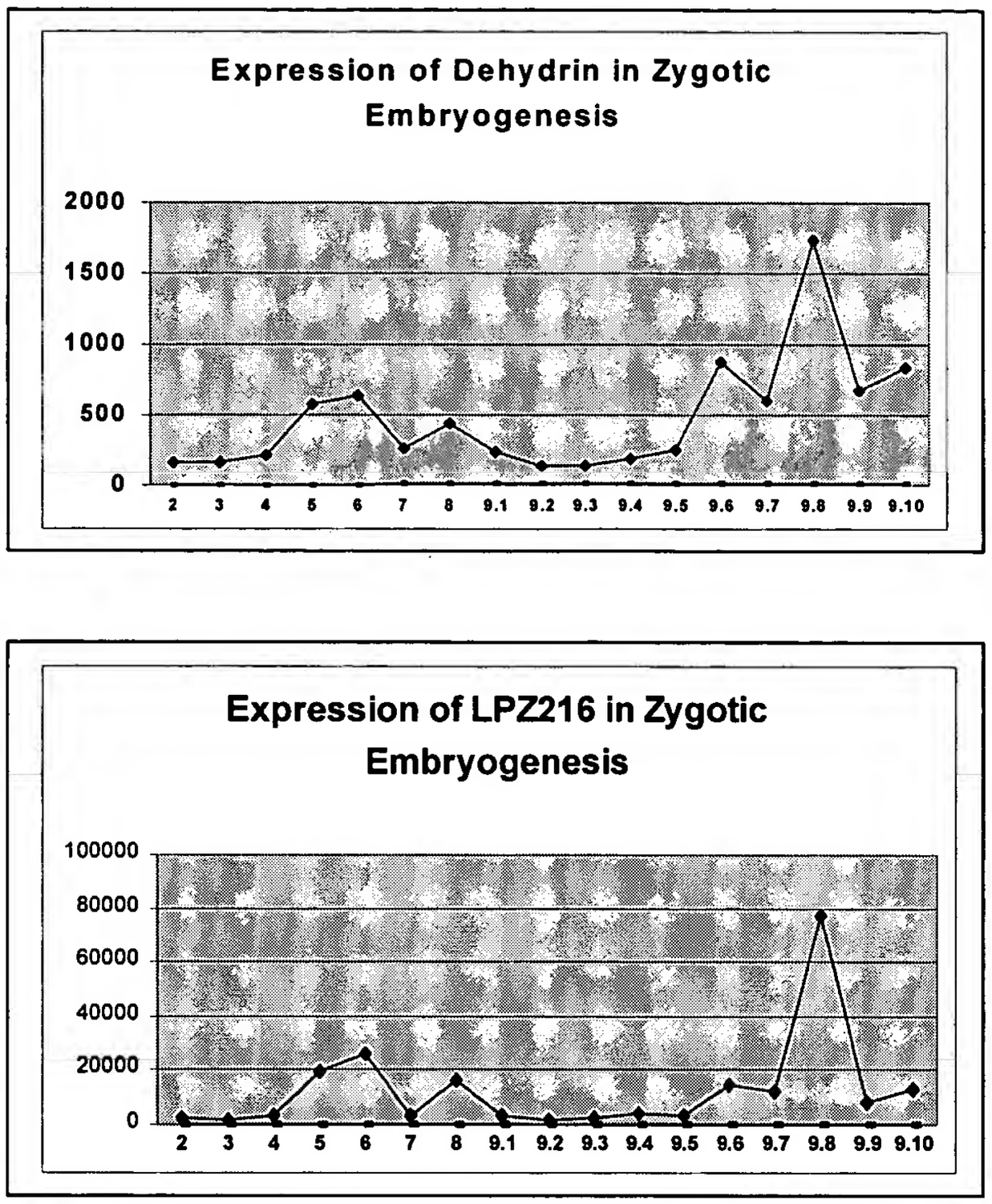


Figure 4. Graphical representation of hybridization of 'dehydrin' and LPZ-216 cDNA probes to total RNA isolated from zygotic embryos of loblolly pine. Five micrograms of RNA was loaded on a slot blot and hybridized with one of the cDNA probes. The hybridization signals were measured by a Fuji BAS-1000 Imaging system and signals were quantified using the associated software. The membrane was then stripped of probe and re-probed with labeled 26S rDNA to determine the equivalence of loading. These signals from this hybridization were captured and used to normalize the signals from dehydrin and LPZ-216.

FIGURE 5

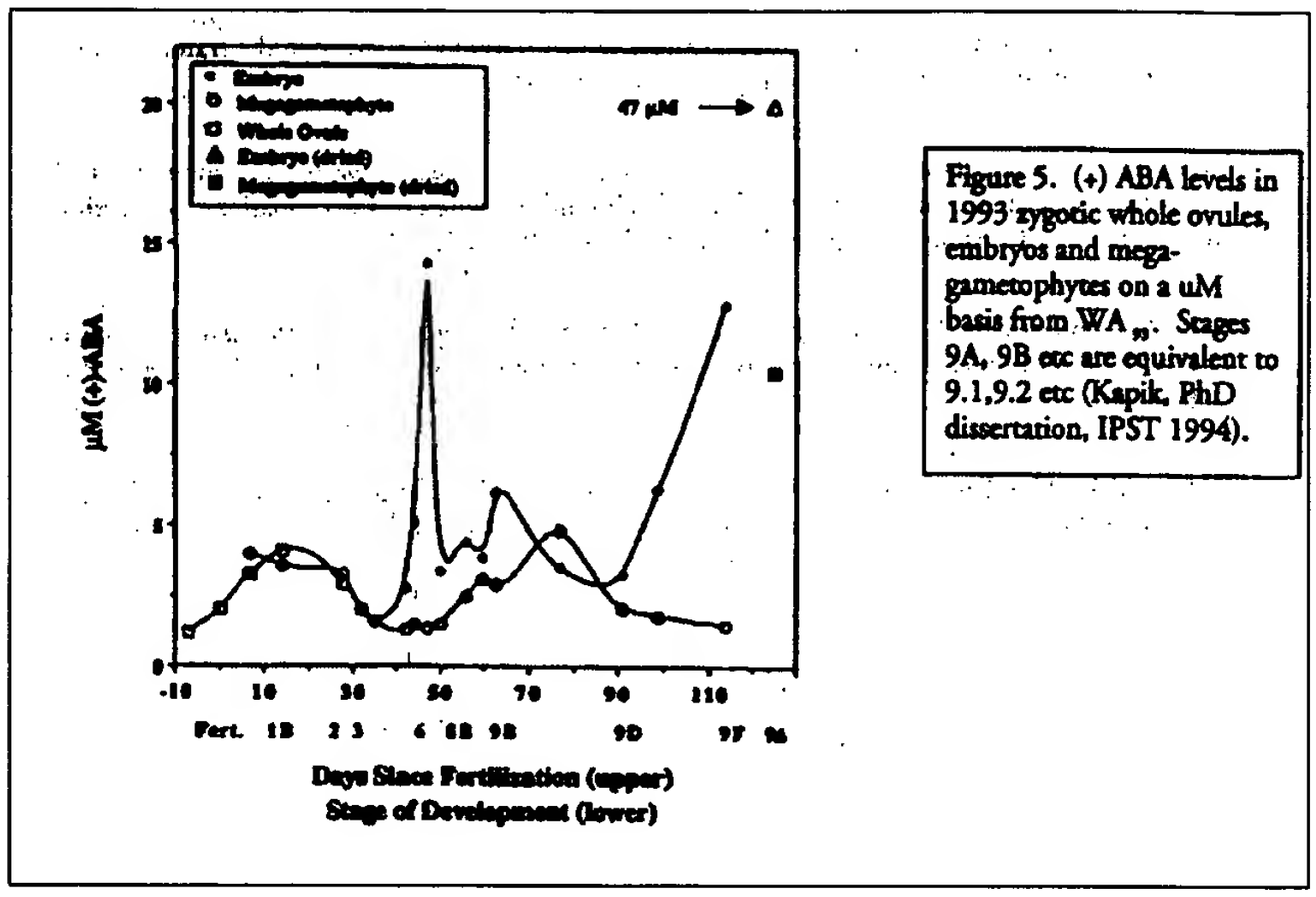


Figure 5. (+) ABA levels in 1993 zygotic whole ovules, embryos and mega-gametophytes on a uM basis from WA $_{99}$. Stages 9A, 9B etc are equivalent to 9.1,9.2 etc (Kapik, PhD dissertation, IPST 1994).

Figure 5. Determination of ABA concentration of loblolly pine embryos as described more fully by Kapik et al., *Tree Physiology* 15:485-490 (1995)

FIGURE 6

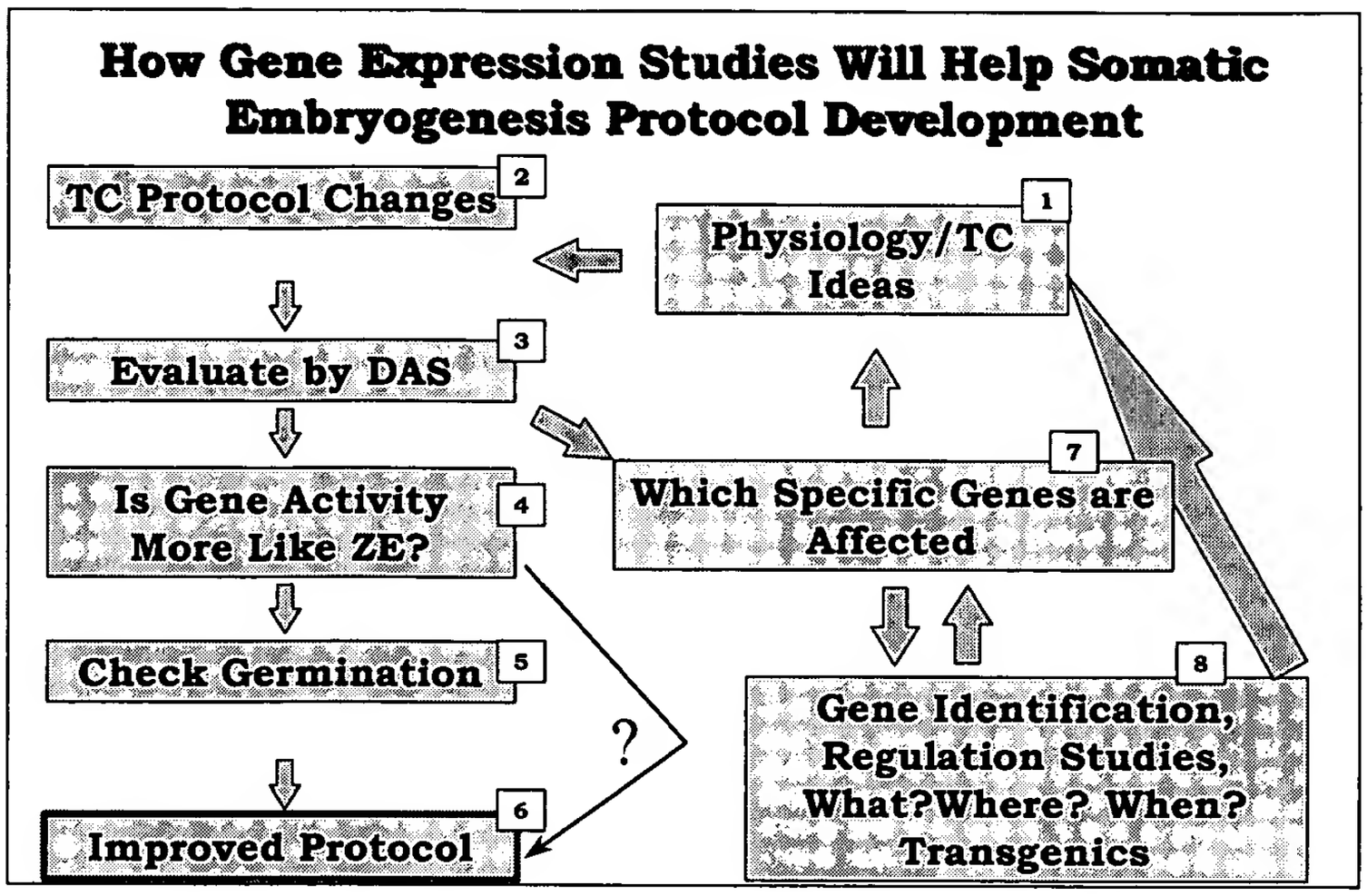


Figure 6. Scheme showing use of gene studies to improve somatic embryogenesis. **TC = Tissue Culture, DAS = DNA Array Southern (an expression monitoring technique), ZE = Zygotic (Natural) Embryo**

FIGURE 7

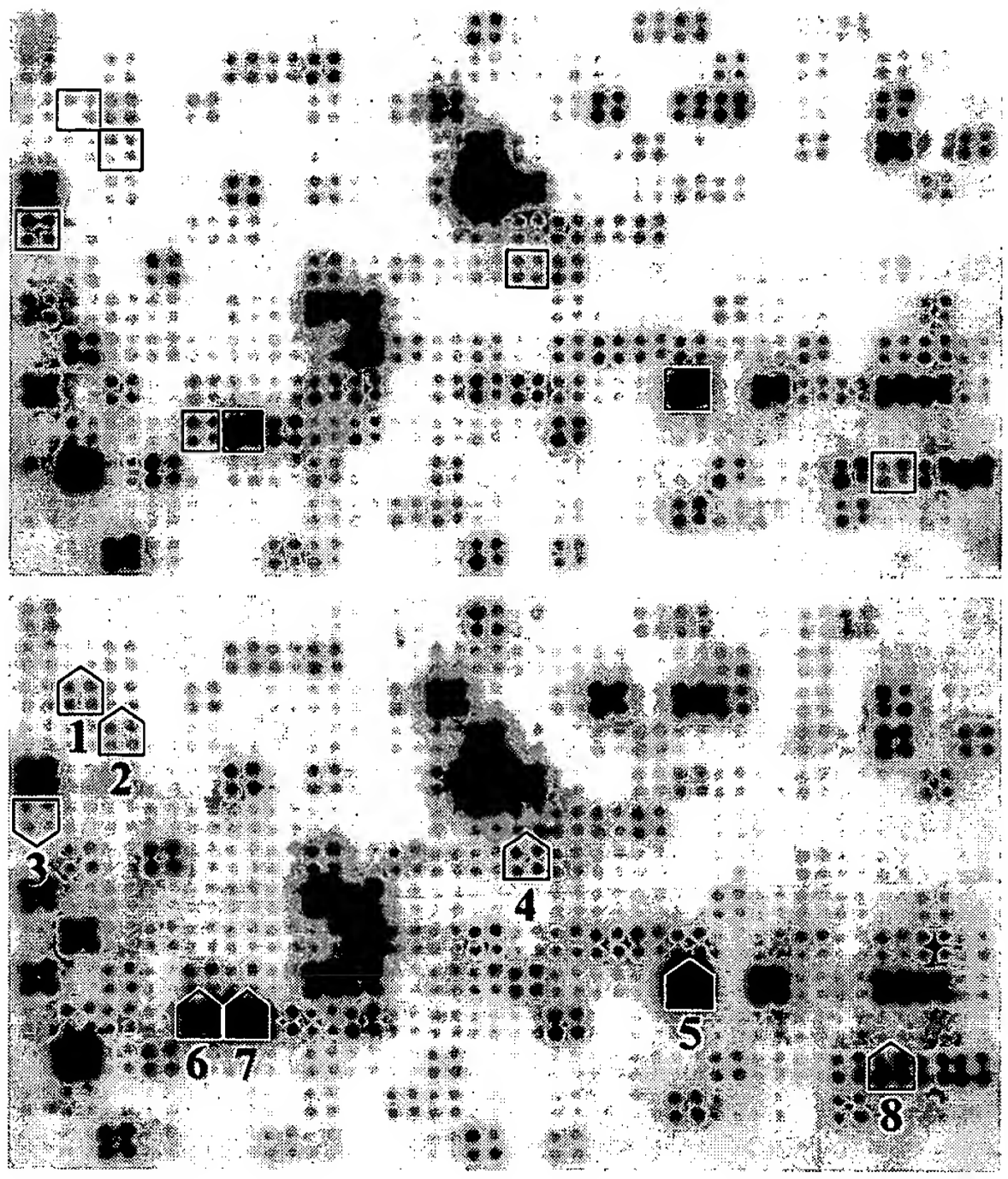


Figure 7. Detection of gene expression by high density array Southern hybridization for loblolly pine genotype 333 after 12 weeks on two maturation media. Top, 5.2 mg/L ABA; bottom 10 mg/L ABA. Arrows up indicate increased gene expression in the 10 mg ABA treatment; arrow down, expression lower in 10 mg ABA treatment. Squares in top panel mark the corresponding spots marked in the bottom panel. Gene 1 (LPS-064), expression is usually higher in ZE than in SE; 2 (LPS-092) expressed in late ZE; 3 (LPZ-049) is starch synthase, higher level in ZE; 4 (LPZ-091) LMW heat shock protein, found in late stage ZE; 5 (LPZ-202) lea gene (late embryo abundant); 6 (LPZ-215) higher level in late ZE; 7 (LPZ-216) lea gene; 8 (LPZ-270) 70S heat shock protein, found in late ZE. A lower level of #3 means a decreased synthesis of starch in 10 mg ABA treatment. All the others bring the expression closer to ZE.

FIGURE 8

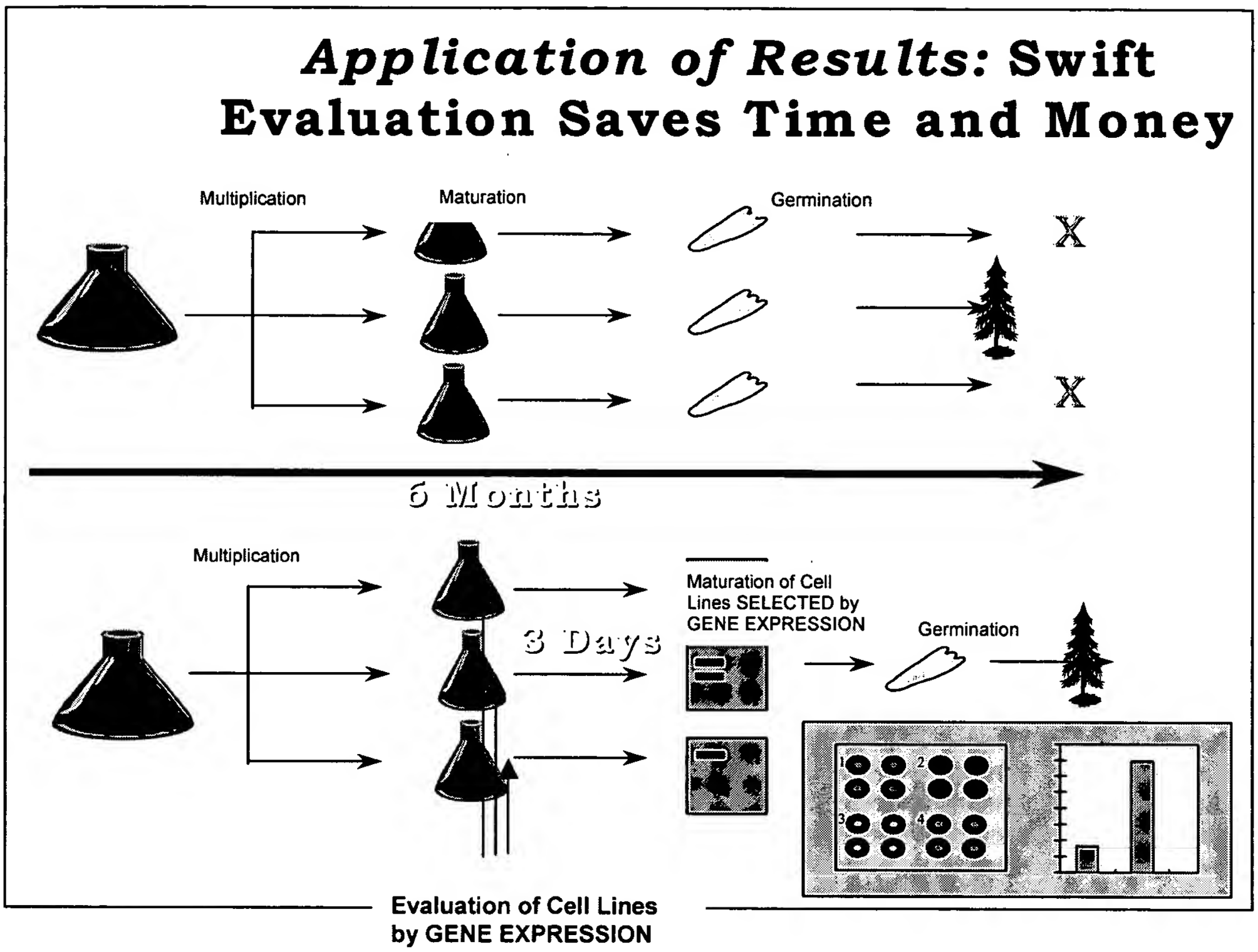
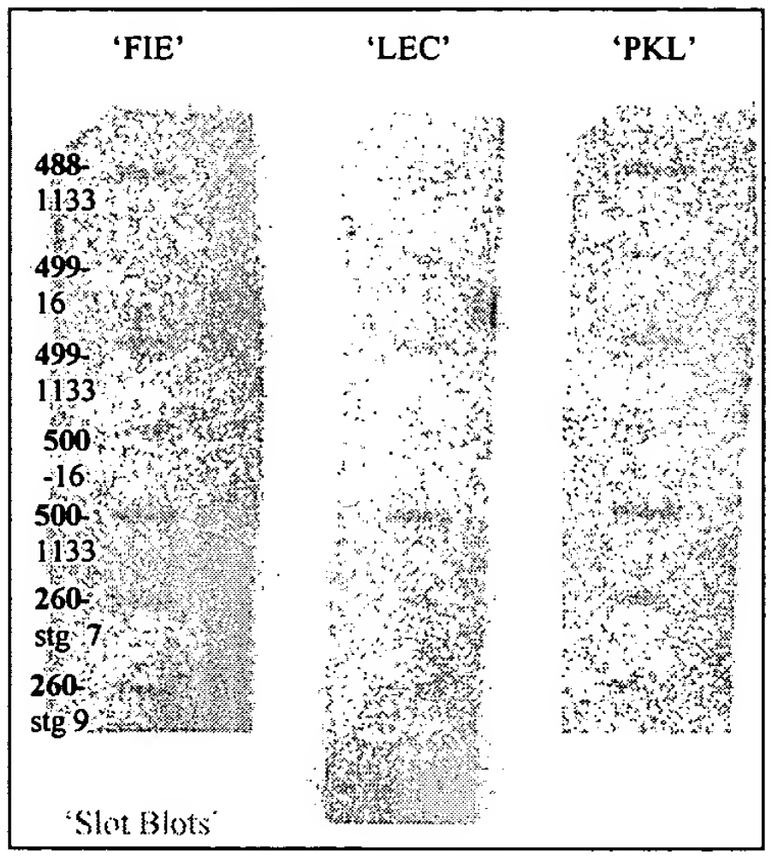


Figure 8. Application of results.

FIGURE 9

A.



B.

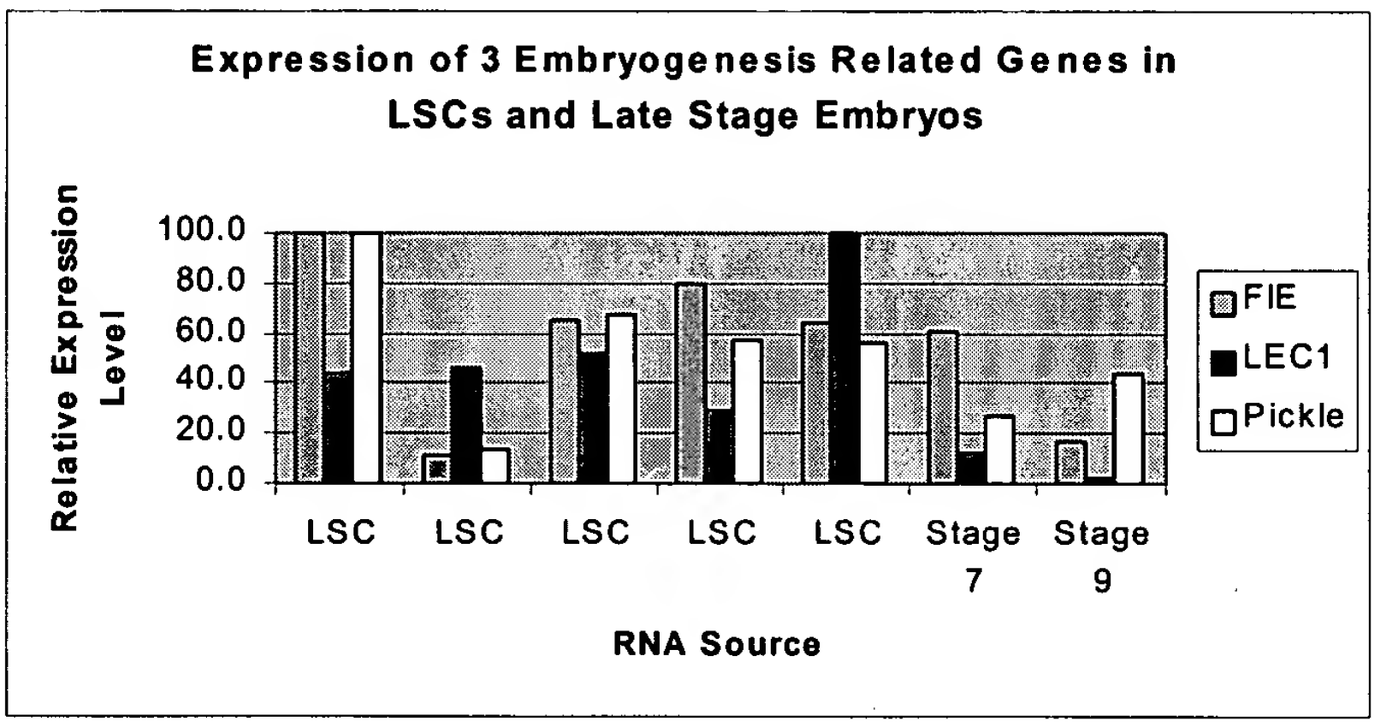


Figure 9. a. Image of RNA slot blot probed with pine cDNA clones bearing similarity to the 'fie' 'lec' or 'pkl' genes from Arabidopsis thaliana. Two micrograms of Loblolly Pine RNA, extracted from either liquid suspension culture (somatic embryos, stage1 &2) or from somatic embryos stage 7 or stage 9, were blotted using a slot blot manifold (Hoeffer ScientificInsturments, San Francisco) according to manufacturers instructions onto Hybond^{TM} N+ (Amersham Pharmacia Biotech, Piscataway, NJ, USA) and UV crosslinked. Each of the three membranes contains identical amounts of the same RNA. The numbers in bold on the left hand side of the images refer to the genotype of the cell line, the numbers below the genotype refer to the media in which embryos were cultured. B. Quantification of the signals shown in panel 9a. Blots were exposed to a phosphorimaging plate for 10 minutes. Screens were read with a BAS1800 (software v1.0) and images were manipulated with ImageGauge (v2.54) (Fuji Photo Film Co., Ltd., Kanagawa, Japan).

FIGURE 10

GGGCACAAAGCTCCGCAGCCTGAGCGAGCGTCATTAGCTTGTCAGTCGGAACCAT

TACCCCTTTCCTCTTCGCTGGCTAGCGAATGATAGGGAATGCTAGCCAGCGAACAA

GATTAGAGCACAGAAAGTATA**GCCAGCGAATCAACAGCATAAC**AACTTAGAGATTTCTTGCAT

TCCCCAGACGGTATCAAGTCATAGTGGAGAATAATCATAATAAGATTTGTGAAAATG

TTTGTGTAGATTAATGTGTAAAATTCAATCCAT**CAACCATGAAGTGAAGTGCATTC**CGTTTTTAA

ATGTTTATTGTATTTGAATGAATAAACAGTTTACACGCGAAAATCCCTACTTTATGTG

CGTACAAACTATGATTTTTTTGCAGTATATAAAAGTTTCCACTATCGTAATTATTTTC

CAGATCCGTCTTCTTAACAACCCGATTTCCTAGCATCCATCTGCGTGGAATAAATCT

ATTGAATTATTAACCCTTGTGATTGGCTAAAAAAAAAA

Figure 10. Sequence of LP2-3 differential display fragment, 507 nucleotides, clone LPS-097.

FIGURE 11

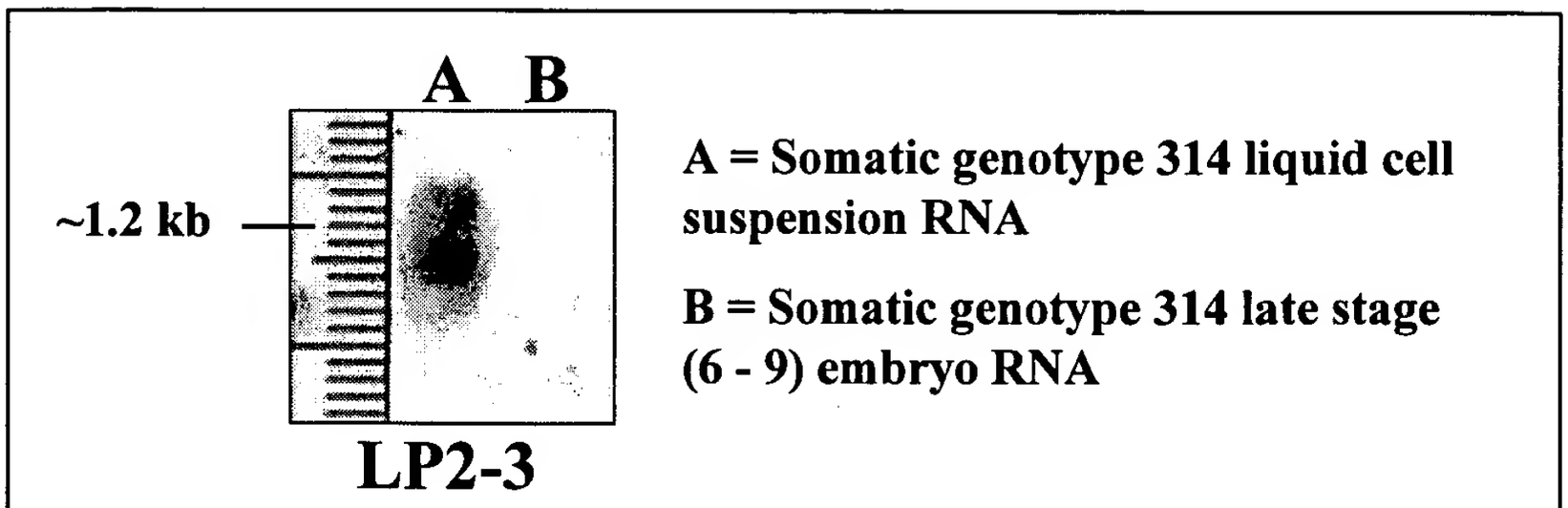
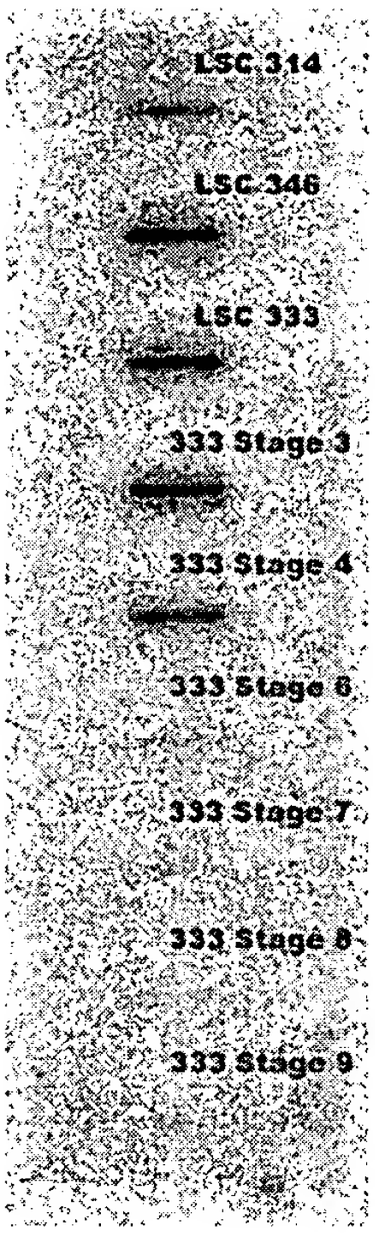
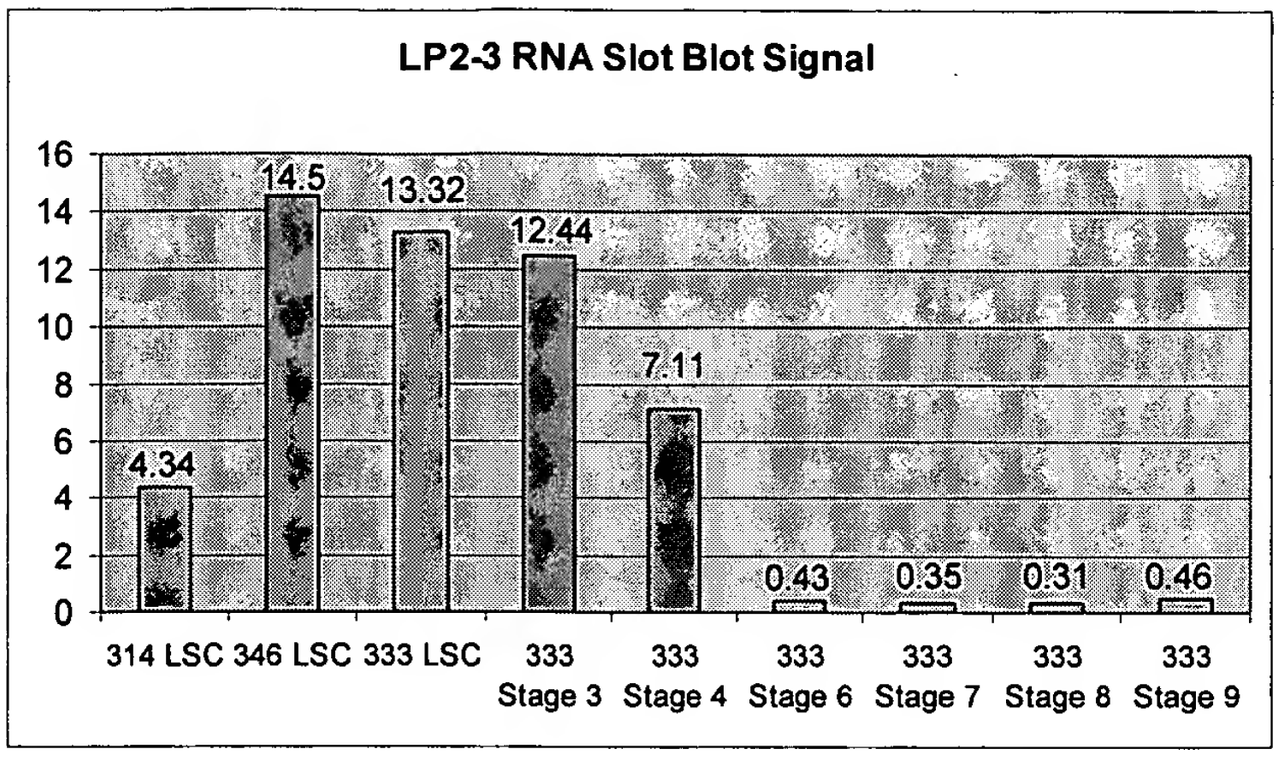


Figure 11. Expression of LP2-3 Gene: Northern Blot of total RNA isolated from Liquid Suspension Culture (Stages 1-3) and Late Stage (Stage 9) Loblolly Pine Somatic Embryos (Pullman & Webb 1994).

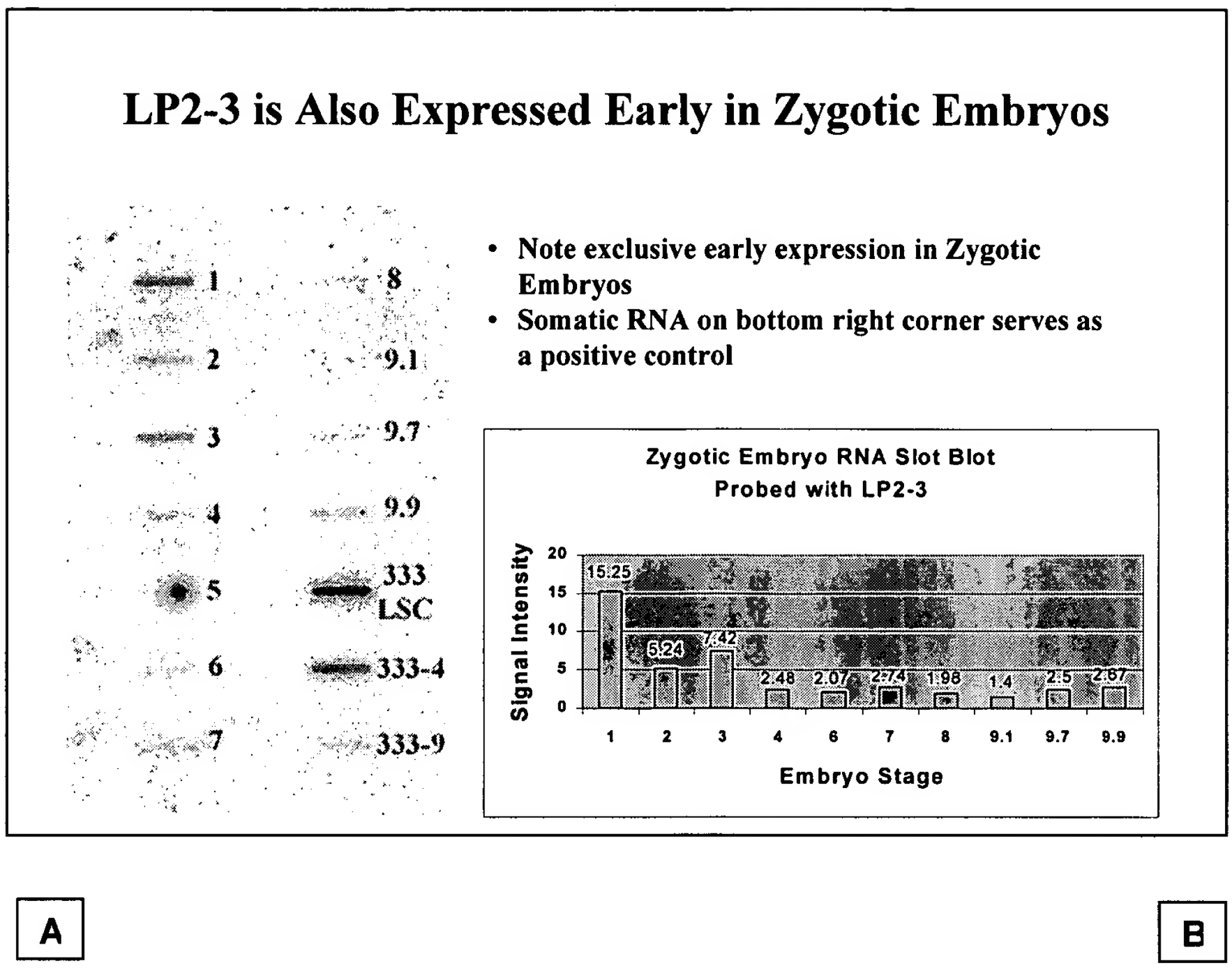
FIGURE 12





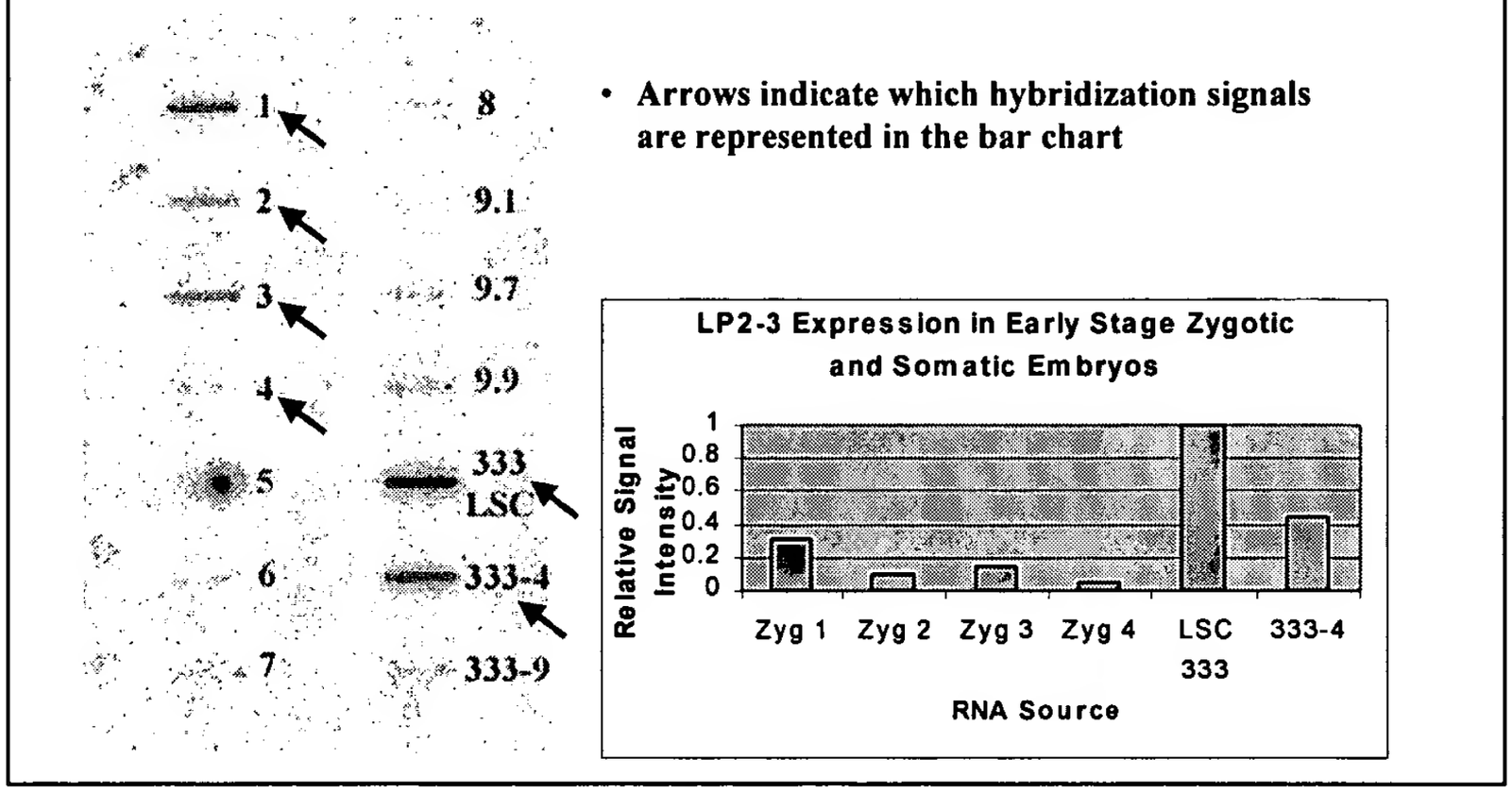
Figures 12A & 12B. Image (1A) and quantification (1B) of a total RNA slot blot probed with an LP2-3-specific probe. For each somatic embryo tissue (liquid suspension culture (LSC) genotypes 314, 346, and 333, and genotype 333 stages 3, 4, 6, 7, 8, and 9) two micrograms of total RNA was attached at each position on the membrane. This blot shows that LP2-3 mRNA is most abundant in early stage somatic embryos, especially when they are in the liquid multiplication medium, and decreases rapidly as embryos begin to mature on maturation medium. It is also apparent that when comparing genotypes, there is variability in LP2-3 abundance in LSC.

FIGURE 13



Figures 13A & 13B. Image (A) and quantification (B) of a total RNA slot blot probed with an LP2-3-specific probe. Isolation of zygotic embryos used in this experiment. From June to September 1996, open-pollinated cones were collected from Union Camp mother tree UC5-1036 were packed on ice and shipped overnight to IPST. Seeds were removed from cones, cracked with a hemostat, and dissected with scalpel and forceps. From each seed the intact ovule was extracted and the megametophyte was sliced open. Embryos were removed, visually judged for stage of development (Pullman & Webb 1994), plunged into liquid nitrogen and stored (20 embryos per 2 mL cryogenic vial (Nalgene Cat. No. 5000)) at -70°C. For somatic embryos, liquid suspension tissue (LSC) was collected, dried by squeezing gently in miracloth (Behring Diagnostics), plunged into liquid nitrogen, and stored at -70°C. Similarly, later stage somatic embryos were plucked from culture, assessed for stage of development, plunged into liquid nitrogen, and stored in vials of 20 to 25 embryos at -70°C.

FIGURE 14



Figures 14A & 14B. Image (A) is as shown in Fig. 13A. The quantified expression of early stage zygotic embryos compared to early stage somatic embryos shown in Fig. B